

## PATENT ABSTRACTS OF JAPAN

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## (54) SKIN CARE PREPARATION

## (57)Abstract:

PROBLEM TO BE SOLVED: To provide a skin care preparation characterized by comprising an extract from a plant of the genus Symplocos.

SOLUTION: This skin care preparation is characterized by comprising the extract from the plant of the genus Symplocos. Furthermore, the extract from the plant of the genus Symplocos has excellent active oxygen scavenging actions, hyaluronidase inhibitory, elastase inhibitory, collagenase inhibitory and tyrosinase inhibitory actions and is stable. The skin care preparation has high stability and exhibits excellent aging preventing and bleaching actions.

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DETAILED DESCRIPTION

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## [Detailed Description of the Invention]

[0001]

[Field of the Invention]The extract of HAINOKI group vegetation is contained in this invention. Therefore, it is related with the skin external preparations excellent in aging prevention and whitening actions.

[0002]

[Description of the Prior Art]In recent years, as a factor which oxidizes a biogenic substance, a free radical and active oxygen are taken up and the adverse effect poses a problem. A free radical and active oxygen are produced in the living body, and a bridge is decomposed or constructed in body tissues, such as collagen, and oil and fat are oxidized, and it is said that the peroxy lipid which does an obstacle to a cell is built. It is thought that such an obstacle causes aging of the wrinkles of skin and Hari falling.

The method of blending the anti-oxidant from which a free radical and active oxygen are removed with one of the methods of preventing aging is known.

Conventionally, ascorbic acid (vitamin C), tocopherol (vitamin E), 3,5-tert-butyl-4-hydroxytoluene (BHT), superoxide dismutase (SOD), etc. have been used for the free radical elimination agent used for the purpose of aging prevention.

[0003]Although the skin consists of epidermis, dermis, and subcutaneous tissue, dermis is very important for structure maintenance of the skin especially, and Hari of the skin is maintained by the dermis connective tissue formed from hyaluronic acid, elastin, collagen, etc. It is thought that wrinkles and sagging of the skin occur as a result which this connective tissue loses a shrinkage force and loses elasticity further.

[0004]If ultraviolet rays are equivalent to the skin, matrix metallo protease, such as elastase and collagenase, will be activated. It is said that these enzymes promote wrinkles and sagging

of the skin by decreasing the elastin and collagen which are the basic components of dermis. Reducing Hari of the skin and causing aging of wrinkles etc. is known by activating hyaluronidase at the time of inflammation and carrying out depolymerize of the hyaluronic acid of polymers.

[0005]In recent years, many cosmetics which prevent wrinkles, sagging, etc. of this skin are known, and retinoic acid, alpha-hydroxy acid, retinol, etc. are reported as that active principle. However, as for these active principles, there is a problem in skin irritation or stability. Blending hyaluronidase, elastase, and collagenase inhibitor with one of the methods of preventing wrinkles and sagging is known. However, HAINOKI group vegetation was not examined as a vegetable raw material with the inhibitory action of these enzymes.

[0006]The melanin generation cell to which the pigmentation of the skin generally looked at by silverfish, a freckle, suntan, etc. exists in the skin by the abnormalities of hormone or stimulus of ultraviolet rays generates a melanin superfluously, and it is considered to be the cause that this deposits in the skin. The method of controlling superfluous generation of melanin to one of the methods of preventing such pigmentation is known. Conventionally, treatment which applies hydroquinone, ascorbic acid (vitamin C), etc. externally has been carried out to the therapy of pigmentation.

[0007]

[Problem(s) to be Solved by the Invention]It cannot be said that SOD used for the purpose of the aging prevention of the skin or antioxidation is unstable, pharmaceutical-preparation-izing is difficult for it, and the effect of vitamin E is [ SOD ] enough. As for BHT which is a synthetic compound, there is a problem in safety, and since loadings have restriction, not a chemical composition but few stable natural raw materials of side effects are desired. Similarly, it is preferred to aging prevention to have safe and stable hyaluronidase, elastase, and collagenase inhibitory action. Since the ascorbic acid used by carrying out a whitening agent has a fault of being easy to decompose temporally, it is extremely stable similarly and skin external preparations of the natural product origin which was excellent in the effect are desired.

[0008]Skin external preparations which were safe, were excellent in stability and were excellent in aging prevention and whitening actions from the above thing are desired.

[0009]

[Means for Solving the Problem]It found out this invention persons having an active oxygen elimination operation, hyaluronidase inhibition, elastase inhibition and collagenase inhibition excellent in an extract of HAINOKI group vegetation, and tyrosinase inhibitory action, as a result of inquiring wholeheartedly, and excelling also in stability according to such a situation. Skin external preparations containing the extract are safe, and are stable, and it finds out excelling in aging prevention and whitening actions, and came to complete this invention.

[0010]For HAINOKI group vegetation (department of HAINOKI) used for this invention. India native SHIMPU localized-oxidation-of-silicon RAKEMOSA (*Symplocos racemosa*), and a U.S. native one, [ SHIMPU localized-oxidation-of-silicon TINKU doria ] AMASHIBA distributed over Taiwan from Amayoshi islands (*Symplocos microcalyx*), HAINOKI widely distributed over western part of Japan (*Symplocos myrtacea*), KUROKI (*Symplocos lucida*), motorcycle a clo (*Symplocos prunifolia*), Shiloh Bayh (*Symplocos lancifolia*), can subROUNOKI (*Symplocos theophrastaefolia*), etc. can be mentioned.

[0011]With an extract of HAINOKI group vegetation used for this invention, it extracts from some or the entire plants of a plant body, such as a leaf of a plant body, a stem, a bark, a flower, a fruit, and a root. What is preferably produced by extracting from a leaf, a stem, and a bark of a plant body is good. The extraction method in particular may not be limited, for example, may carry out heating extraction, and may carry out ordinary temperature extraction.

[0012]as the solvent to extract – water and lower alcohol (methanol.) Ethanol, 1-propanol, 2-propanol, 1-butanol, liquefied polyhydric alcohol (a 1,3-butylene glycol.), such as 2-butanol Ketone (acetone, methyl ethyl ketone, etc.), such as propylene glycol and glycerin. Acetonitrile, ester species, hydrocarbon (ethyl acetate, butyl acetate, etc.), and ether (hexane, heptane, a liquid paraffin, etc.) (ethyl ether, a tetrahydrofuran, propyl ether, etc.) are mentioned. It is desirable, polar solvents, such as water, lower alcohol, and liquefied polyhydric alcohol, are at best especially preferred, and water, ethanol, a 1,3-butylene glycol, and propylene glycol are good. A kind may also mix two or more sorts and may use these solvents.

[0013]The above-mentioned extract may be used with an extracted solution, if needed, may carry out decolorization by concentration, dilution and filtration treatment, activated carbon, etc., deodorization treatment, etc., and may be used. Concentration hardening by drying, spray drying, freeze-drying, etc. may be processed, and an extracted solution may be used as a dry matter.

[0014]Within limits which may use the above-mentioned extract for skin external preparations of this invention as it is, and do not spoil an effect of an extract. Ingredients, such as oil and fat which are an ingredient used for external preparations, lows, hydrocarbon, fatty acid, alcohols, ester species, a surface-active agent, metallic soap, a pH adjuster, an antiseptic, perfume, a moisturizer, a granular material, an ultraviolet ray absorbent, a thickener, coloring matter, an antioxidant, a whitening agent, and a chelating agent, can be blended.

[0015]Can use skin external preparations of this invention for both cosmetics quasi drugs and drugs, and as the dosage forms, For example, what is applied to the skins, such as face toilet, cream, a milky lotion, gel, aerosols, essence, a pack, a detergent, baths, foundation, dusting powder, a lip stick, ointment, and cataplasms, is mentioned.

[0016]Loadings of the above-mentioned extract used for this invention are converted into a solid to the skin external-preparations whole quantity of this invention, and 0.001 to 10% of the

weight of its combination is preferably good 0.0001% of the weight or more. It is hard to desire effect sufficient at less than 0.0001 % of the weight. Enhancement of an effect is [ that it is hard to accept ] uneconomical when it blends exceeding 10 % of the weight. What is necessary is to add in the middle of manufacture beforehand, to consider workability, and just to choose suitably about a method of addition.

[0017]

[Example]Next, in order to explain this invention in detail, the example of manufacture of the extract used for this invention as an example, the example of a formula of this invention, and the example of an experiment are given, but this invention is not limited to this. As for the part of the loadings shown in an example, % shows weight % for a weight section.

[0018]Example of manufacture 1 After adding purified water 400mL to the dry matter 20g of the bark of hot water extract SHIMPU localized-oxidation-of-silicon RAKEMOSA of SHIMPU localized-oxidation-of-silicon RAKEMOSA and extracting at 95-100 °C for 2 hours, it filtered and the filtrate was condensed, it freeze-dried and 4.0g of hot water extracts of SHIMPU localized-oxidation-of-silicon RAKEMOSA were obtained.

[0019]Example of manufacture 2 After adding the ethanol 1L to the dry matter 100g of the bark of ethanol extract SHIMPU localized-oxidation-of-silicon RAKEMOSA of SHIMPU localized-oxidation-of-silicon RAKEMOSA and extracting for seven days at ordinary temperature, it filtered, concentration hardening by drying of the filtrate was carried out, and 5.8g of ethanol extracts of SHIMPU localized-oxidation-of-silicon RAKEMOSA were obtained.

[0020]Example of manufacture 3 Purified water 200mL and 1,3-butylene-glycol 200mL are added to the dry matter 20g of the bark of 50% 1,3-butylene-glycol extract SHIMPU localized-oxidation-of-silicon RAKEMOSA of SHIMPU localized-oxidation-of-silicon RAKEMOSA, After extracting for seven days at ordinary temperature, it filtered and 380g of 50% 1,3-butylene-glycol extracts of SHIMPU localized-oxidation-of-silicon RAKEMOSA were obtained.

[0021]example of manufacture 4 SHIMPU localized-oxidation-of-silicon TINKU -- purified water 400mL to the leaf of hot water extract SHIMPU localized-oxidation-of-silicon RAKEMOSA of doria, and the dry matter 20g of fruits, [ add and ] filtering, condensing the filtrate and freeze-drying, after extracting at 95-100 °C for 2 hours -- SHIMPU localized-oxidation-of-silicon TINKU -- 3.0g of hot water extracts of doria were obtained.

[0022]Example of manufacture 5 After adding the ethanol 1L to the dry matter 100g of the bark of ethanol extract HAINOKI of HAINOKI and extracting for seven days at ordinary temperature, it filtered, concentration hardening by drying of the filtrate was carried out, and 5.8g of ethanol extracts of HAINOKI were obtained.

[0023]Example of manufacture 6 Purified water 200mL and 1,3-butylene-glycol 200mL are added to the leaf of 50% 1,3-butylene-glycol extract HAINOKI of HAINOKI, and the dry matter 20g of a bark, After extracting for seven days at ordinary temperature, it filtered and 380g of

50%1,3-butylene-glycol extracts of HAINOKI were obtained.

[0024]

Example 1 Face toilet formula Loadings Hot water extract (example 1 of manufacture) of 1. SHIMPU localized-oxidation-of-silicon RAKEMOSA 0.1 copy 2.1,3-butylene glycol 8.0 3. glycerin 2.04. xanthan gum 0.02 5. citrate 0.01. 6. Sodium acid citrate 0.1 7. ethanol 5.0 8. methyl parahydroxybenzoate 0.1 9. polyoxyethylene hydrogenated castor oil (40E.O.) 0.110. perfume The [manufacturing method] ingredients 1-6, and 11 which set the whole quantity to 100 with optimum dose 11. purified water, The ingredients 7-10 are dissolved in homogeneity, respectively, both are mixed and filtered, and it is considered as a product.

[0025]Comparative example 1 In the conventional face toilet example 1, what transposed the hot water extract of SHIMPU localized-oxidation-of-silicon RAKEMOSA to purified water was used as conventional face toilet.

[0026]

example 2 cream formula Loadings Ethanol extract (example 2 of manufacture) of 1. SHIMPU localized-oxidation-of-silicon RAKEMOSA 0.05 copy 2. squalane 5.5 3. olive oil 3.0 4. stearic acid 2.0 5. yellow bees wax 2.0 6. myristic acid octyldodecyl . 3.5 7. polyoxyethylene cetyl ether (20E.O.) 3.0 8. behenyl alcohol 1.5 9. glyceryl monostearate 2.510. perfume 0.111. methyl parahydroxybenzoate 0.212. ethyl p-hydroxybenzoate . 0.0513.1,3-butylene glycol With 8.514. purified water, the heating and dissolving of the [manufacturing method] ingredients 2-9 which set the whole quantity to 100 are carried out, and it mixes, keeps at 70 \*\*, and is considered as an oil phase. The heating and dissolving of the ingredient 1, and 11-14 are carried out, and it mixes, keeps at 75 \*\*, and is considered as the aqueous phase. It cools adding, emulsifying and stirring the aqueous phase to an oil phase, and the ingredient 10 is added at 45 \*\*, and also it cools to 30 \*\*, and is considered as a product.

[0027]Comparative example 2 In the conventional cream example 2, what transposed the ethanol extract of SHIMPU localized-oxidation-of-silicon RAKEMOSA to purified water was used as conventional cream.

[0028]

example 3 milky-lotion formula Loadings Hot water extract (example 1 of manufacture) of 1. SHIMPU localized-oxidation-of-silicon RAKEMOSA 0.001 copy 2. squalane 5.0 3. olive oil 5.0 4. jojoba oil 5.0 5. cetanol 1.5 6. glyceryl monostearate . 2.0 7. polyoxyethylene cetyl ether (20E.O.) 3.0 8. polyoxyethylene sorbitan monooleate 2.0 (20E.O.) 9. Perfume 0.110. propylene glycol 1.011. glycerin 2.012. methyl parahydroxybenzoate With 0.213. purified water, the heating and dissolving of the [manufacturing method] ingredients 2-8 which set the whole quantity to 100 are carried out, and it mixes, keeps at 70 \*\*, and is considered as an oil phase. The heating and dissolving of the ingredient 1, and 10-13 are carried out, and it mixes, keeps at 75 \*\*, and is considered as the aqueous phase. It cools

adding, emulsifying and stirring the aqueous phase to an oil phase, and the ingredient 9 is added at 45 \*\*, and also it cools to 30 \*\*, and is considered as a product.

[0029]

Example 4 gel formula Loadings 1. SHIMPU localized-oxidation-of-silicon RAKEMOSA 1.0 copy 50% 1,3-butylene-glycol extract (example 3 of manufacture) 2. ethanol 5.0 3. methyl parahydroxybenzoate . 0.1 4. polyoxyethylene hydrogenated castor oil (60E.O.) 0.1 5. perfume Optimum dose 6. 1,3-butylene glycol 5.0 7. glycerin 5.0 8. xanthan gum 0.1 9. carboxyvinyl polymer . 0.2 10. Potassium hydrate The [manufacturing method] ingredients 2-5 which set the whole quantity to 100 with 0.2 11. purified water, the ingredient 1, and 6-11 are dissolved in homogeneity, respectively, both are mixed, and it is considered as a product.

[0030]

example 5 pack formula Loadings 1. SHIMPU localized-oxidation-of-silicon TINKU -- hot water extract (example 4 of manufacture) of doria 0.1 copy Ethanol extract (example 5 of manufacture) of 2. HAINOKI 0.1 3. polyvinyl alcohol 12.0 4. ethanol . 5.0 5. 1,3-butylene glycol 8.0 6. methyl parahydroxybenzoate 0.2 7. polyoxyethylene hydrogenated castor oil (20E.O.) 0.5 8. citrate 0.1 9. sodium acid citrate 0.3 10. perfume With optimum dose 11. purified water, the whole quantity. The [manufacturing method] ingredients 1-11 set to 100 are dissolved uniformly, and it is considered as a product.

[0031]

Example 6 Foundation formula Loadings Ethanol extract (example 5 of manufacture) of 1. HAINOKI 1.0 copy 2. stearic acid 2.4 3. polyoxyethylenesorbitan monostearate 1.0 (20E.O.) 4. Polyoxyethylene cetyl ether (20E.O.). 2.0 5. cetanol 1.0 6. liquefied lanolin . 2.0 7. liquid paraffin 3.0 8. myristic acid isopropyl 6.5 10. carboxymethylcellulose sodium 0.1 11. bentonite 0.5 12. propylene glycol 4.0 13. triethanolamine . 1.1 14. Methyl parahydroxybenzoate 0.2 15. titanium dioxide 8.0 16. talc 4.0 17. red ocher 1.0 18. yellow oxide of iron 2.0 19. perfume The heating and dissolving of the [manufacturing method] ingredients 2-9 which set the whole quantity to 100 with optimum dose 20. purified water are carried out, and it keeps at 80 \*\*, and is considered as an oil phase. The ingredient 10 is well swollen for the ingredient 20, then the ingredient 1, and 11-14 are added, and it mixes uniformly. The ingredients 15-18 which carried out grinding mixing with the grinder are added to this, and it agitates by a homomixer, keeps at 75 \*\*, and is considered as the aqueous phase. Cooling, adding the ingredient 19 at 45 \*\* in addition, stirring an oil phase to this aqueous phase, and stirring, it cools to 30 \*\* and is considered as a product.

[0032]

Example 7 Baths formula Loadings Hot water extract (example 1 of manufacture) of 1. SHIMPU localized-oxidation-of-silicon RAKEMOSA 5.0 copies 2. sodium bicarbonate 50.0 3.

yellow No. 202 (1) Optimum dose 4. perfume Optimum dose With 5. sodium sulfate, the whole quantity. The [manufacturing method] ingredients 1-5 set to 100 are mixed uniformly, and it is considered as a product.

[0033]

example 8 ointment formula Loadings 1. SHIMPU localized-oxidation-of-silicon TINKU -- hot water extract (example 4 of manufacture) of doria 0.01 copy 50%1,3-butylene-glycol extract of 2. HAINOKI 0.5 (example 6 of manufacture)

3. Polyoxyethylene cetyl ether (30E.O.). 2.0 4. glyceryl monostearate 10.0 5. liquid paraffin 5.0 6. cetanol 6.0 7. methyl parahydroxybenzoate 0.1 8. propylene glycol 10.0 The [manufacturing method] ingredients 3-6 which set the whole quantity to 100 with 9. purified water. Heating and dissolving are carried out, and it mixes, keeps at 70 \*\*, and is considered as an oil phase. The heating and dissolving of the ingredients 1 and 2, and 7-9 are carried out, and it mixes, keeps at 75 \*\*, and is considered as the aqueous phase. Adding, emulsifying and stirring the aqueous phase to an oil phase, it cools to 30 \*\* and is considered as a product.

[0034]Next, in order to explain the effect of this invention in detail, the example of an experiment is given.

[0035]Example of experiment 1 The elimination operation of superoxide which is a kind of active oxygen was measured using the examples 1, 2, 4, and 5 of active oxygen elimination operation manufacture as a sample. Superoxide dismutase (SOD) was used as positive control. In order to check the stability of a sample, the sample was saved for two weeks at 40 \*\*, and it measured similarly.

[0036]sample aqueous solution 0.1mL of each concentration -- the color reagent (a 0.24mM nitroblue tetrazolium.) of 0.45mL The 0.1M phosphate buffer solution containing a 0.4mM xanthin; added the enzyme liquid (0.1 U/mL xanthine oxidase and 0.1M phosphate buffer solution;pH8.0) of pH8.0 and 0.45mL, it was made to react for 20 minutes at 37 \*\*, and JIHORUMAZAN was produced. After adding reaction stop solution (69mM sodium-dodecyl-sulfate solution) 1.0mL to this solution, the absorbance in the wavelength of 560 nm was measured. The inhibitory action of each sample was computed by the erase rate searched for from the following formula. Purified water was used for contrast instead of the sample, and the 0.1M phosphate buffer solution (pH 8.0) was used instead of the tyrosinase as blank. Erase rate (%) =  $[1 - (C-D)/(A-B)] \times 100A$ : The absorbance at 560 nm of contrast (O. D.560) B: contrast -- blank O.D.560C: -- O.D.560D: of a sample -- a sample -- blank O.D.560 -- the test result of these was shown in Table 1. As a result, although, as for SOD, the active oxygen elimination operation decreased greatly by preservation for two weeks at 40 \*\*, the extract of HAINOKI group vegetation did not have change in an elimination operation. The active oxygen elimination operation whose extract of HAINOKI group vegetation was stable and which was excellent from the above thing was shown.



[0037]

[Table 1]

表 1. 活性酸素消去試験結果

試 料	活性酸素消去率 (%)		
	0. 1 m g / m L	0. 5 m g / m L	5 U / m L
製造例 1 の抽出物	57 (59)	>95 (>95)	
製造例 2 の抽出物	60 (57)	>95 (>95)	
製造例 4 の抽出物	50 (48)	>95 (95)	
製造例 5 の抽出物	45 (45)	90 (91)	
SOD (緑性対照)			70 (19)

( ) 内は、40℃、2週間保存した試料を用いた測定値

[0038]Example of experiment 2 Method to which the Morgan-Elson method was applied for hyaluronidase inhibitory action using the examples 1, 2, 4, and 5 of hyaluronidase inhibitory action manufacture as a sample It measured according to a food hygienics magazine, and [31, 3] (1990). Namely, 0.1M acetic acid buffer (pH 4.0) 175microL is added to a sample solution, As it furthermore became [ enzyme activity / of hyaluronidase ] 0.06 mg/mL about the compound 48/80 of 0.4 mg/mL and an activator in the concentration of 400 U/mL and hyaluronic acid, after adjusting the whole quantity to 500microL, the hyaluronidase reaction was carried out for 40 minutes at 37 \*\*. The p-dimethylaminobenzaldehyde reagent was made to add and color after a reaction, and the absorbance at 585 nm was measured. The inhibitory action of each sample was computed at the inhibition rate called for from the following formula. Purified water was used for contrast instead of the sample, and purified water was used instead of hyaluronidase as blank.

Inhibition rate (%) =  $[1 - (C - D) / (A - B)] \times 100A$ : The absorbance at 585 nm of contrast (O. D.585) B: O.D.585C of a contrast blank : O.D.585 of the O.D.585D:sample blank of a sample [0039] These experimental results were shown in Table 2. As a result, the extract of HAINOKI group vegetation showed the outstanding hyaluronidase inhibitory action.

[0040]

[Table 2]

表 2. ヒアルロニダーゼ阻害試験結果

試 料	ヒアルロニダーゼ阻害率 (%)	
	0. 1 m g / m L	0. 5 m g / m L
製造例 1 の抽出物	33	63
製造例 2 の抽出物	40	65
製造例 4 の抽出物	30	51
製造例 5 の抽出物	22	42

[0041]Example of experiment 3 Elastase inhibitory action was measured using the examples 1, 2, 4, and 5 of elastase inhibitory action manufacture as a sample. 50microL Add 0.02 mg/mL elastase Type 3 (product made from a sigma), and trischloride buffer solution (pH 8.0) to sample solution 50muL as enzyme liquid using a microplate. 100microL N-Succinyl-Ala-Ala-

Ala-rho-nitroanilide (product made from a sigma) and 0.2M trischloride buffer solution of 0.45 mg/mL (pH 8.0) as a substrate solution After mixing in addition, It was made to react for 1 hour and 37 \*\* of absorbances at 415 nm were measured. The inhibitory action of each sample was computed at the inhibition rate called for from the following formula. Purified water was used for contrast instead of the sample, and 0.2M trischloride buffer solution (pH 8.0) was used instead of elastase as blank.

Inhibition rate (%) =  $[1-(C-D)/(A-B)] \times 100A$ : The absorbance at 415 nm of contrast (O. D.415) B: O.D.415C of a contrast blank : O.D.415 of the O.D.415D:sample blank of a sample [0042] These experimental results were shown in Table 3. As a result, the extract of HAINOKI group vegetation showed the outstanding elastase inhibitory action.

[0043]

[Table 3]

表3. エラスターゼ阻害試験結果

試 料	エラスターゼ阻害率 (%)
製造例1の抽出物	75
製造例2の抽出物	78
製造例4の抽出物	73
製造例5の抽出物	70

試験濃度は、固形分として1mg/mL

[0044]Example of experiment 4 Collagenase inhibitory action was measured using the examples 1, 2, 4, and 5 of collagenase inhibitory action manufacture as a sample. 50microl Add collagenase Type 4 (product made from a sigma) solution of 0.1 mg/mL to sample solution 50muL as enzyme liquid using a microplate. It is Pz-peptide ( ) of 0.39 mg/mL as a substrate solution. [ Pz-Pro-Leu-Gly-Pro-D-Arg-OH and ] After mixing moreover and making 37 \*\* of the product made from a sigma and trischloride buffer solution containing 20mM calcium chloride (pH 7.1) react for 3 minutes, 25mM citrate 1mL was added and the reaction was stopped. Ethyl acetate 5mL was added and extracted and the absorbance of 320 nm was measured for the ethyl acetate layer. The inhibitory action of each sample was computed at the inhibition rate called for from the following formula. Purified water was used for contrast instead of the sample, and the trischloride buffer solution containing 20mM calcium chloride (pH 7.1) was used instead of collagenase as blank.

Inhibition rate (%) =  $[1-(C-D)/(A-B)] \times 100A$ : The absorbance at 320 nm of contrast (O. D.320) B: O.D.320C of a contrast blank : O.D.320 of the O.D.320D:sample blank of a sample [0045] These experimental results were shown in Table 4. As a result, the extract of HAINOKI group vegetation showed the outstanding collagenase inhibitory action.

[0046]

[Table 4]

表4. コラグナーゼ阻害試験結果

試 料	コラグナーゼ阻害率 (%)
製造例1の抽出物	> 95
製造例2の抽出物	> 95
製造例4の抽出物	> 95
製造例5の抽出物	90

試験濃度は、固形分として1 mg/mL

[0047]Example of experiment 5 Tyrosinase inhibitory action was measured using the examples 1, 2, 4, and 5 of tyrosinase inhibitory action manufacture as a sample. Ascorbic acid was used as positive control. In order to check the stability of a sample, the sample was saved for two weeks at 40 \*\*, and it measured similarly.

[0048]After a tyrosinase inhibition examination adds sample-solution 0.2mL and L-tyrosine solution (0.2 mg/mL) 1mL, and the Mac vein's buffer solution (pH 6.8) 0.6mL to a test tube, Tyrosinase solution 0.2mL of 1,000 U/mL could be added, and it mixed, and it was made to react for 3 minutes and 37 \*\* of absorbances at 475 nm were measured. The inhibitory action of each sample was computed at the inhibition rate called for from the following formula. Purified water was used for contrast instead of the sample, and purified water was used instead of the tyrosinase as blank.

Inhibition rate (%) =  $[1-(C-D)/(A-B)] \times 100A$ : The absorbance at 475 nm of contrast (O. D.475) B: O.D.475C of a contrast blank : O.D.475 of the O.D.475D:sample blank of a sample [0049] These experimental results were shown in Table 5. As a result, the extract of HAINOKI group vegetation showed the outstanding tyrosinase inhibitory action. When the ascorbic acid used for positive control was saved for two weeks at 40 \*\*, tyrosinase inhibitory action fell, but the extract of HAINOKI group vegetation did not have change in inhibitory action. The tyrosinase inhibitory action whose extract of HAINOKI group vegetation was stable and which was excellent from the above thing was shown.

[0050]

[Table 5]

表5. チロシナーゼ阻害試験結果

試 料	チロシナーゼ阻害率 (%)	
	0. 1 mg/mL	1 mg/mL
製造例1の抽出物	22 (22)	74 (73)
製造例2の抽出物	30 (31)	78 (79)
製造例4の抽出物	21 (20)	69 (66)
製造例5の抽出物	18 (19)	57 (58)
アスコルビン酸 (陽性対照)		65 (30)

( ) 内は、40℃、2週間保存した試料を用いた測定値

[0051]Example of experiment 6 The use examination for one month was done for 30 women (21-46 years old) using the face toilet of use examination 1 Example 1, the cream of Example 2 and the conventional face toilet of the comparative example 1, and the conventional cream of

the comparative example 2. The wrinkles of skin and the improvement effect of sagging were judged by the questionnaire after use.

[0052] These test results were shown in Table 6. As a result, the skin external preparations containing the extract of HAINOKI group vegetation showed the improving action of outstanding wrinkles and sagging. During the test period, skin troubles did not have one person, either and were satisfactory also in safety. It was satisfactory also about degradation of a formula ingredient.

[0053]

[Table 6]

表 6. 使用試験 1

試 験 品	シワ、タルミの改善作用の判定 (人)		
	改善した	やや改善した	改善されない
実施例 1 の化粧水	13	13	4
比較例 1 の従来化粧水	2	14	14
実施例 2 のクリーム	17	12	1
比較例 2 の従来クリーム	2	15	13

[0054] Example of experiment 7 The use examination for one month was done using the face toilet of use examination 2 Example 1, the cream of Example 2 and the conventional face toilet of the comparative example 1, and the conventional cream of the comparative example 2 for 15 women (21-46 years old) who worry about silverfish and a freckle. The improvement effect of silverfish and a freckle was judged by the questionnaire after use.

[0055] These test results were shown in Table 7. As a result, the skin external preparations containing the extract of HAINOKI group vegetation showed the improving action of outstanding silverfish and freckle. During the test period, skin troubles did not have one person, either and were satisfactory also in safety. It was satisfactory also about degradation of a formula ingredient.

[0056]

[Table 7]

表 7. 使用試験 2

試 験 品	シミ、ソバカスの改善作用の判定 (人)		
	改善した	やや改善した	改善されない
実施例 1 の化粧水	11	16	3
比較例 1 の従来化粧水	0	15	15
実施例 2 のクリーム	13	15	2
比較例 2 の従来クリーム	1	15	14

[0057] When the use examination was similarly done about Examples 3-8, improving actions, such as outstanding wrinkles, sagging, silverfish, and a freckle, were shown.

[0058]

[Effect of the Invention] From the above thing, the extract of the HAINOKI group vegetation of

this invention has outstanding active oxygen elimination operation, hyaluronidase inhibition, elastase inhibition and collagenase inhibition, and tyrosinase inhibitory action, and was excellent also in stability. The skin external preparations containing these extracts showed the aging prevention which was safe and was excellent, and whitening actions.

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[Translation done.]